

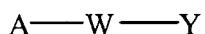
Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1 1 (currently amended): A nucleic acid-lipid particle composition for introducing
2 a nucleic acid into a cell, said particle composition comprising:

3 (a) a nucleic acid-lipid particle comprising a cationic lipid, a conjugated lipid that
4 inhibits aggregation of particles, and a nucleic acid, wherein said nucleic acid is encapsulated in
5 ~~the~~ a lipid bilayer of said nucleic acid-lipid particle, and wherein said conjugated lipid that
6 inhibits aggregation of particles is a member selected from the group consisting of a PEG-lipid,
7 an ATTA-lipid and a cationic-polymer-lipid conjugate having the formula



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9 wherein:

10 A is a lipid moiety;

11 W is a hydrophilic polymer; and

12 Y is a polycationic moiety; and

13 (b) an endosomal membrane destabilizer, wherein said endosomal membrane
14 destabilizer is Ca^{++} ion.

1 2 (original): The nucleic acid-lipid particle composition of claim 1, wherein said
2 endosomal membrane destabilizer is outside said nucleic acid-lipid particle.

1 3 (original) The nucleic acid-lipid particle composition of claim 1, wherein said
2 endosomal membrane destabilizer is both outside and inside said nucleic acid-lipid particle.

4 (cancelled)

1 5 (original): The nucleic acid-lipid particle composition of claim 4, wherein the
2 concentration of Ca^{++} ion is from about 0.1 mM to about 100 mM.

1 6 (original): The nucleic acid-lipid particle composition of claim 5, wherein the
2 concentration of Ca^{++} ion is from about 1 mM to about 20 mM.

1 7 (original): The nucleic acid-lipid particle composition of claim 1, wherein said
2 particle has a median diameter of less than about 150 nm.

1 8 (original): The nucleic acid-lipid particle composition of claim 1, wherein said
2 cationic lipid is a member selected from the group consisting of N,N-dioleoyl-N,N-
3 dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide
4 (DDAB), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(1-
5 (2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), and N,N-dimethyl-2,3-
6 dioleoyloxy)propylamine (DODMA), and combinations thereof.

1 9 (original): The nucleic acid-lipid particle composition of claim 1, wherein said
2 particle further comprises an additional noncationic lipid.

1 10 (original): The nucleic acid-lipid particle composition of claim 9, wherein
2 said noncationic lipid is selected from the group consisting of DOPE, POPC, and EPC.

1 11 (original): The nucleic acid-lipid particle composition of claim 1, wherein
2 said particle comprises a functional group that facilitates Ca^{++} ion chelation.

1 12 (original): The nucleic acid-lipid particle composition of claim 1, wherein
2 said conjugated lipid that inhibits aggregation of particles has the formula



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4 wherein:

5 A is a lipid moiety;
6 W is a hydrophilic polymer; and
7 Y is a polycationic moiety.

1 13 (original): The nucleic acid-lipid particle composition of claim 12, wherein W
2 is a polymer selected from the group consisting of PEG, polyamide, polylactic acid, polyglycolic
3 acid, polylactic acid/polyglycolic acid copolymers and combinations thereof, said polymer
4 having a molecular weight of about 250 to about 7000 daltons.

1 14 (original): The nucleic acid-lipid particle composition of claim 12, wherein Y
2 has at least 4 positive charges at a selected pH.

1 15 (original): The nucleic acid-lipid particle composition of claim 12, wherein Y
2 is a member selected from the group consisting of lysine, arginine, asparagine, glutamine,
3 derivatives thereof and combinations thereof.

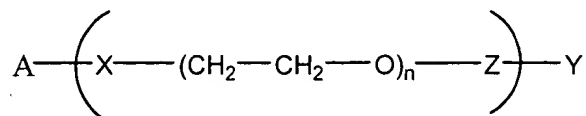
1 16 (original): The nucleic acid-lipid particle composition of claim 12, wherein A
2 is a member selected from the group consisting of a diacylglycerol moiety, a dialkylglycerol
3 moiety, a N-N-dialkylamino moiety, a 1,2-diacyloxy-3-aminopropane moiety and a 1,2-dialkyl-
4 3-aminopropane moiety.

1 17 (original): The nucleic acid-lipid particle composition of claim 12, wherein W
2 is PEG.

1 18 (original): The nucleic acid-lipid particle composition of claim 12, wherein W
2 is a polyamide polymer.

1 19 (original): The nucleic acid-lipid particle composition of claim 12, wherein W
2 has a molecular weight of about 250 to about 2000 daltons.

20 (original): The nucleic acid-lipid particle composition of claim 17, having the general structure of Formula II:



II

wherein

X is a member selected from the group consisting of a single bond or a functional group covalently attaching said lipid to at least one ethylene oxide unit;

Z is a member selected from the group consisting of a single bond or a functional group covalently attaching said at least one ethylene oxide unit to a cationic group; and

n is an integer having a value of between about 6 to about 50.

21 (original): The nucleic acid-lipid particle composition of claim 20, wherein

X is a member selected from the group consisting of a single bond, phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho, phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate, amido, thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

22 (original): The nucleic acid-lipid particle composition of claim 20, wherein

Z is a member selected from the group consisting of a single bond, phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho, phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate, amido, thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

23 (original): The nucleic acid-lipid particle composition of claim 20, wherein

A is a diacylglycerol moiety;

X is phosphoethanolamido;

Z is NR, wherein R is a hydrogen atom; and

5 Y is a member selected from the group consisting of about 1 to about 10 basic
6 amino acids or derivatives thereof.

1 24 (original): The nucleic acid-lipid particle composition of claim 23, wherein
2 A is a diacylglycerol moiety having 2 fatty acyl chains, wherein each acyl chain
3 is independently between 2 and 30 carbons in length and is either saturated or has varying
4 degrees of saturation.

1 25 (original): The nucleic acid-lipid particle composition of claim 23, wherein
2 Y is a member selected from the group consisting of lysine, arginine, asparagine,
3 glutamine, derivatives thereof and combinations thereof.

1 26 (original): The nucleic acid-lipid particle composition of claim 23, wherein
2 A is a diacylglycerol moiety having 2 fatty acyl chains, wherein each acyl chain
3 is a saturated C-18 carbon chain; and
4 Y is a cationic group having 4 lysine residues or derivatives thereof.

1 27 (original): The nucleic acid-lipid particle composition of claim 1, wherein
2 said conjugated lipid that inhibits aggregation of particles is a PEG-lipid

1 28 (original): The nucleic acid-lipid particle composition of claim 27, wherein
2 said PEG-lipid is PEG-ceramide.

1 29 (original): The nucleic acid-lipid particle composition of claim 28, wherein
2 the ceramide of said PEG-ceramide comprises a fatty acid group having about 8 to about 20
3 carbon atoms.

1 30 (original): The nucleic acid-lipid particle composition of claim 28, wherein
2 said PEG-lipid is PEG-phosphatidylethanolamine.

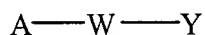
1 31 (original): The nucleic acid-lipid particle composition of claim 1, wherein
2 said conjugated lipid that inhibits aggregation of particles is an ATTA-lipid.

1 32 (original): The nucleic acid-lipid particle composition of claim 1, wherein
2 said nucleic acid is selected from the group consisting of a plasmid, an antisense oligonucleotide,
3 and a ribozyme.

1 33 (currently amended): A method of introducing a nucleic acid into a cell, said
2 method comprising:

3 contacting said cell with a nucleic acid-lipid particle composition, said particle
4 composition comprising:

5 (a) a nucleic acid-lipid particle comprising a cationic lipid, a conjugated lipid that
6 inhibits aggregation of particles, and a nucleic acid, wherein said nucleic acid is encapsulated in
7 ~~the~~ a lipid bilayer of said nucleic acid-lipid particle, and wherein said conjugated lipid that
8 inhibits aggregation of particles is a member selected from the group consisting of a PEG-lipid,
9 an ATTA-lipid and a cationic-polymer-lipid conjugate having the formula



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11 wherein:

12 A is a lipid moiety;

13 W is a hydrophilic polymer; and

14 Y is a polycationic moiety; and

15 (b) an endosomal membrane destabilizer, wherein said endosomal membrane
16 destabilizer is Ca^{++} ion.

1 34 (original): The method of introducing a nucleic acid into a cell of claim 33,
2 wherein said endosomal membrane destabilizer is outside said nucleic acid-lipid particle.

1 35 (original): The method of introducing a nucleic acid into a cell of claim 33,
2 wherein said endosomal membrane destabilizer is Ca^{++} ion.

1 36 (original): The method of introducing a nucleic acid into a cell of claim 35,
2 wherein the concentration of Ca^{++} ion is from about 0.1 mM to about 100 mM.

1 37 (original): The method of introducing a nucleic acid into a cell of claim 36,
2 wherein the concentration of Ca^{++} ion is from about 1 mM to about 20 mM.

1 38 (original): The method of introducing a nucleic acid into a cell of claim 33,
2 wherein said particle has a median diameter of less than about 150 nm.

1 39 (original): The method of introducing a nucleic acid into a cell of claim 33,
2 wherein said cationic lipid is a member selected from the group consisting of N,N-dioleoyl-N,N-
3 dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide
4 (DDAB), N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N-(1-
5 (2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), and N,N-dimethyl-2,3-
6 dioleoyloxy)propylamine (DODMA), and combinations thereof.

1 40 (original): The method of introducing a nucleic acid into a cell of claim 33,
2 wherein said particle further comprises an additional noncationic lipid.

1 41 (original): The method of introducing a nucleic acid into a cell of claim 40,
2 wherein said noncationic lipid is selected from the group consisting of DOPE, POPC, and EPC.

1 42 (original): The method of introducing a nucleic acid into a cell of claim 33,
2 wherein said particle comprises a functional group that facilitates Ca^{++} ion chelation.

1 43 (original): The method of introducing a nucleic acid into a cell of claim 33,
2 wherein said conjugated lipid that inhibits aggregation of particles has the formula



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wherein:

A is a lipid moiety;

W is a hydrophilic polymer; and

Y is a polycationic moiety.

44 (original): The method of introducing a nucleic acid into a cell of claim 43, wherein W is a polymer selected from the group consisting of PEG, polyamide, polylactic acid, polyglycolic acid, polylactic acid/polyglycolic acid copolymers and combinations thereof, said polymer having a molecular weight of about 250 to about 7000 daltons.

45 (original): The method of introducing a nucleic acid into a cell of claim 43, wherein Y has at least 4 positive charges at a selected pH.

46 (original): The method of introducing a nucleic acid into a cell of claim 43, wherein Y is a member selected from the group consisting of lysine, arginine, asparagine, glutamine, derivatives thereof and combinations thereof.

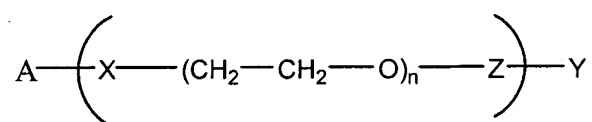
47 (original): The method of introducing a nucleic acid into a cell of claim 43, wherein A is a member selected from the group consisting of a diacylglycerol moiety, a dialkylglycerol moiety, a N-N-dialkylamino moiety, a 1,2-diacyloxy-3-aminopropane moiety and a 1,2-dialkyl-3-aminopropane moiety.

48 (original): The method of introducing a nucleic acid into a cell of claim 43, wherein W is PEG.

49 (original): The method of introducing a nucleic acid into a cell of claim 43, wherein W is a polyamide polymer.

50 (original): The method of introducing a nucleic acid into a cell of claim 43,
wherein W has a molecular weight of about 250 to about 2000 daltons.

51 (original): The method of introducing a nucleic acid into a cell of claim 48,
having the general structure of Formula II:



II

wherein

X is a member selected from the group consisting of a single bond or a functional
group covalently attaching said lipid to at least one ethylene oxide unit;

Z is a member selected from the group consisting of a single bond or a functional
group covalently attaching said at least one ethylene oxide unit to a cationic group; and

n is an integer having a value of between about 6 to about 50.

52 (original): The method of introducing a nucleic acid into a cell of claim 51,
wherein

X is a member selected from the group consisting of a single bond,
phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho,
phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate, amido,
thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

53 (original): The method of introducing a nucleic acid into a cell of claim 51,
wherein

Z is a member selected from the group consisting of a single bond,
phosphatidylethanolamino, phosphatidylethanolamido, phosphoro, phospho,

5 phosphoethanolamino, phosphoethanolamido, carbonyl, carbamate, carboxyl, carbonate, amido,
6 thioamido, oxygen, sulfur and NR, wherein R is a hydrogen or alkyl group.

1 54 (original): The method of introducing a nucleic acid into a cell of claim 51,
2 wherein

3 A is a diacylglycerol moiety;

4 X is phosphoethanolamido;

5 Z is NR, wherein R is a hydrogen atom; and

6 Y is a member selected from the group consisting of about 1 to about 10 basic
7 amino acids or derivatives thereof.

1 55 (original): The method of introducing a nucleic acid into a cell of claim 54,
2 wherein

3 A is a diacylglycerol moiety having 2 fatty acyl chains, wherein each acyl chain
4 is independently between 2 and 30 carbons in length and is either saturated or has varying
5 degrees of saturation.

1 56 (original): The method of introducing a nucleic acid into a cell of claim 54,
2 wherein

3 Y is a member selected from the group consisting of lysine, arginine, asparagine,
4 glutamine, derivatives thereof and combinations thereof.

1 57 (original): The method of introducing a nucleic acid into a cell of claim 54,
2 wherein

3 A is a diacylglycerol moiety having 2 fatty acyl chains, wherein each acyl chain
4 is a saturated C-18 carbon chain; and

5 Y is a cationic group having 4 lysine residues or derivatives thereof.

1 58 (original): The method of introducing a nucleic acid into a cell of claim 33,
2 wherein said conjugated lipid that inhibits aggregation of particles is a PEG-lipid.

1 59 (original): The method of introducing a nucleic acid into a cell of claim 58,
2 wherein said PEG-lipid is PEG-ceramide.

1 60 (original): The method of introducing a nucleic acid into a cell of claim 59,
2 wherein the ceramide of said PEG-ceramide comprises a fatty acid group having about 8 to about
3 20 carbon atoms.

1 61 (original): The method of introducing a nucleic acid into a cell of claim 59,
2 wherein said PEG-lipid is PEG-phosphatidylethanolamine.

1 62 (original): The method of introducing a nucleic acid into a cell of claim 33,
2 wherein said conjugated lipid that inhibits aggregation of particles is an ATTA-lipid.

1 63 (original): The method of introducing a nucleic acid into a cell of claim 33,
2 wherein said nucleic acid is selected from the group consisting of a plasmid, an antisense
3 oligonucleotide, and a ribozyme.

1 64 (original): A method for inducing H_{II} phase structure in a lipid bilayer, said
2 method comprising: contacting said lipid bilayer with an endosomal membrane destabilizer,
3 thereby inducing H_{II} phase structure in a lipid bilayer.

1 65 (original): The method for inducing H_{II} phase structure of claim 64, wherein
2 said lipid bilayer comprises DOPC:DOPE:DOPS:Chol.

1 66 (original): The method for inducing H_{II} phase structure of claim 64, wherein
2 said endosomal membrane destabilizer is Ca⁺⁺ ion.

1 67 (original): The method for inducing H_{II} phase structure of claim 66, wherein
2 Ca⁺⁺ ion acts in concert with low levels of the cationic lipid to trigger H_{II} phase formation.

68 (cancelled)